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MICROSCOPIC OBSERVATIONS OF CRYPTIC POLAR BODY PRODUCTION AND SPIRALIAN ORGANIZATION IN THE EGG OF *ILYANASSA OBSOLETA*

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Abstract

Light microscopy of semi-thin sections and scanning electron microscopy reveal a pit at the animal pole of *Ilyanassa obsoleta* during Meiosis I. The incipient polar body is within the pit. Sections show the spindle at an oblique angle to the egg axis, an early expression of the spiralian organization of this egg. Surface changes on the incipient polar body are similar to those described by others for the polar lobe of *Nassarius reticulatus.*

Introduction

In the marine mud snail *Ilyanassa obsoleta* the egg is ripe at late prophase of Meiosis I and fertilization is internal. The eggs are fertilized in the pallial oviduct, and subsequently surrounded by a viscous fluid, enclosed in capsules (usually in groups of 50–250), and deposited on an external substratum. Normally, the eggs are deposited before maturation is complete, but there is great variability in the time elapsed between fertilization and deposition—in some cases, 24 h or more (Collier, 1981). Thus, the time of deposition is unreliable in determining the stage of development, and selecting just-laid capsules does not insure that eggs pooled from these different capsules are the same fertilization age. Collier (pers. comm.) found that a reliable criterion is the appearance of a dark spot at the animal pole. This spot, which can be seen under low magnification, is found only in the freshest eggs (preceding first polar lobe formation), lessens in intensity after 20–30 minutes, and disappears before the second polar lobe forms.

For many years this dark spot was thought to be the germinal vesicle and its disappearance, the breakdown of that body. Recently, light microscopy of semi-thin sections of this stage have shown differently: in reality the spot is a depression, or pit, at the bottom of which the first polar body is forming. Scanning electron microscopy has confirmed this observation.

Materials and Methods

Adult snails were maintained and freshly laid capsules of fertilized eggs were obtained as described by Collier (1981). Eggs were removed from the capsules and fixed either in (1) a solution of picric acid to saturation and 2% paraformaldehyde in 0.10 *M* NaH_2PO_4 buffer at pH 7.3 (PAF), which is a modification of a procedure of Stefanini *et al.* (1967), for 2 h at room temperature, rinsed in buffer, left in lithium-saturated buffer overnight, and rinsed again in buffer, or (2) in 1% OsO_4 in 0.4 *M* sodium acetate, pH 6.0, for 1 h at room temperature (Burgess, 1977). The PAF

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fixative, which was developed by Stefanini and others (1967) for electron microscopy of the acrosome and plasma membrane of mammalian sperm, as modified here proved to be the fixative of choice for *Ilyanassa* ovary and eggs. The yolk platelets, which are extremely dense and comprise 32% of the egg volume, are more readily and effectively fixed by this rapidly penetrating formula than by glutaraldehyde. For light microscopy osmium tetroxide offers no advantages and significant disadvantages *e.g.,* the density of its staining, interfere with subsequent staining, and other treatments.

For light microscopy, eggs were embedded in glycol methacrylate (Leduc and Bernhard, 1967) and sectioned at 2 μm with glass knives on a Porter-Blum ultramicrotome. Serial sections were placed on a clean glass slide, floated on water, expanded by gentle heat, and dried down. Sections were stained with azure B (Flax and Himes, 1952) and fast green FCF (Himes and Moriber, 1956) and observed using phase contrast and bright light optics.

For electron microscopy, the eggs were critical-point dried, coated with gold palladium, and viewed with an Hitachi 405A scanning electron microscope.

Results

Observations of semi-thin sections of uncleaved *Ilyanassa obsoleta* eggs revealed a vase-shaped depression, or pit, at the animal pole. In profile, the mouth and lower portion of the pit are somewhat flared, with a narrower neck in between. At or near the bottom of the pit the first polar body is emerging. (See Fig. 1.)

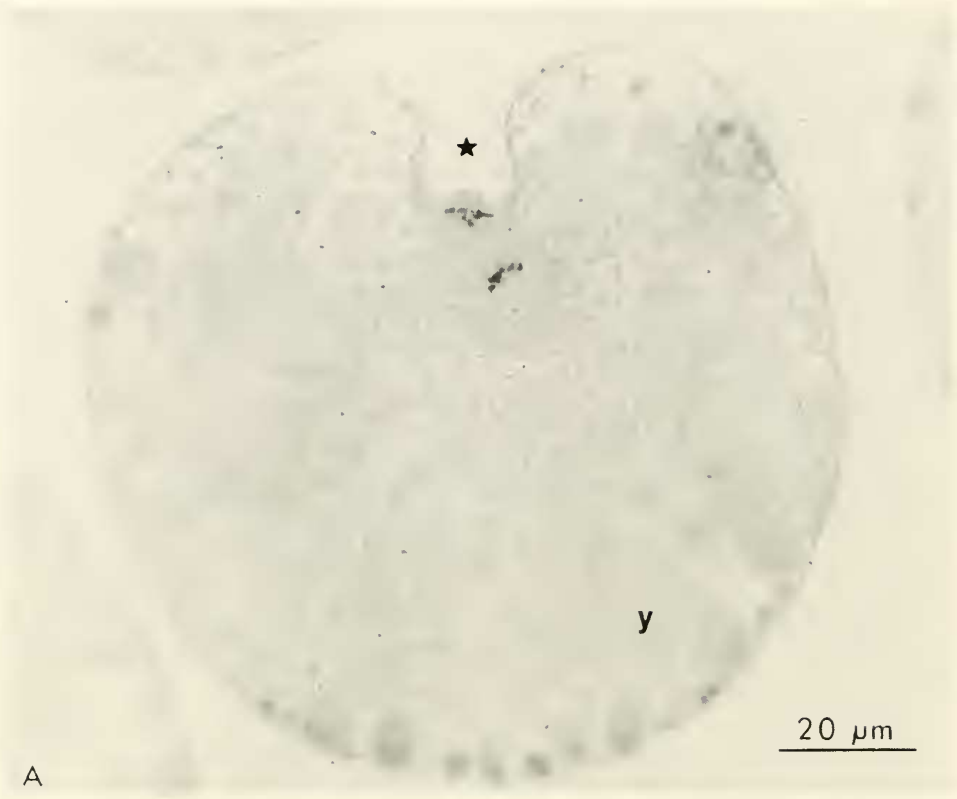
The dimensions of the pit at its greatest development, as determined from sections, are approximately 25 μm across at the mouth, narrowing to approximately 12 μm at the neck, and extending to a depth of 20–23 μm. At this phase the emerging polar body protrudes approximately 2 μm from the bottom of the pit and is thus some 20 μm from the outer surface of the egg.

In the living egg, at relatively low magnifications (~70×) afforded by the dissecting microscope, the pit appears as a dark spot and cannot be discerned as an indentation, even when the egg is rolled so that the animal pole is at an angle to the optical axis. However, scanning electron microscopy of whole eggs confirmed the presence of the pit. The sides of the pit appear steep and vertically ridged and the surface of the incipient polar body is covered with reticulating folds. Clumps of an amorphous, fluffy material are evident on the surface of the egg, but not in the pit. Typically, there are two ridges that extend from the pit walls to the emerging polar body; the other sides of the polar body are more deeply demarcated. (See Fig. 2.)

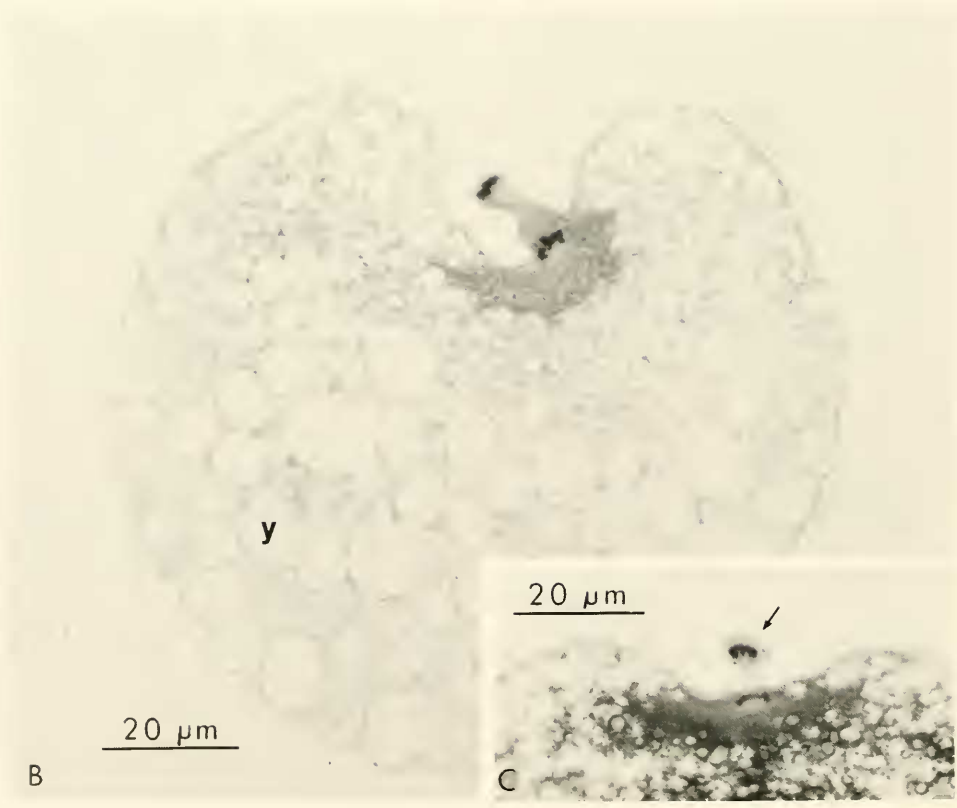
The exact time of the first appearance of the pit is not known. It is certainly postovulation, as semi-thin sections of eggs in the prepallial oviduct show no depression at the animal pole. Artificial insemination experiments (unpub. obs.) provide further information on timing: the first polar lobe appears in most artificially inseminated eggs 60–90 min (24°C) postinsemination. These eggs attained the trefoil stage (*e.g.,* when the first two blastomeres and the third polar lobe are nearly divided three ways) at 240 min postinsemination, on average, and developed into normal veliger larvae.

Observation of sectioned eggs fixed coincident with the darkest spot-appearance show the chromosomes in early telophase I and with no polar lobe. Eggs with less dark spots (regressing pits) were seen to be in mid- to late telophase I, with the beginning of first polar lobe formation occuring at the end of telophase I.

From serial sections through the animal-vegetal axis during Telophase I it is evident that the axis of the meiotic figure is not coincident with the egg's axis, rather it is at an oblique angle. Figure 1.B shows a late Telophase I stage with an angle of approximately 50° between the spindle and the egg's axis.



A



B C

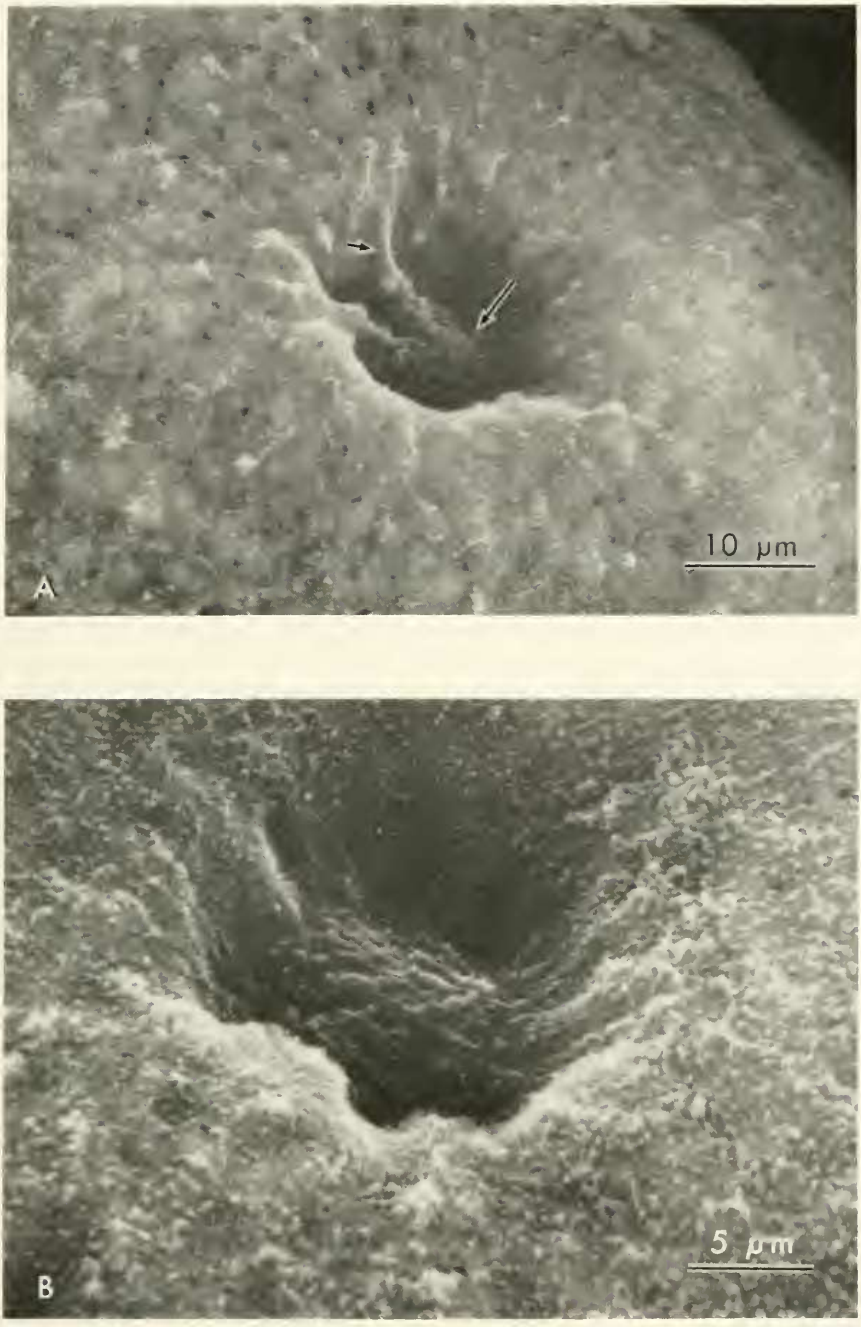


FIGURE 2. Scanning electron micrographs of Meiosis I. Fixed in picric acid-formaldehyde. A. Short arrow indicates ridge; long arrow indicates incipient polar body. B. Note reticulating folds on surface of incipient polar body.

FIGURE 1. Semi-thin sections of Meiosis I. A. Late anaphase. Star indicates pit at animal pole. Y indicates yolk platelet. Fixed in picric acid-formaldehyde; stained with azure B and fast green FCF. B. Late telophase. Fixed in picric acid-formaldehyde; stained with azure B. C. First polar body (at arrow). Fixed in OsO_4; stained with azure B.

The regressing pits show shape changes. First, the neck of the pit widens and the walls become parallel. As telophase is completed, the depression has widened at the mouth and the walls are divergent, giving a blunt conical shape to the depression; the first polar body is nearly detached and extends by a thin thread of membrane halfway, or more, the distance to the surface of the egg. At the same time, the depression has become shallower.

Sections of eggs between first and second polar lobe stages have not been examined. Sections made at second polar lobe formation show, in addition to the first polar body, telophase of Meiosis II occurring in a shallow depression of about 4 μm depth.

Discussion

Others have found alterations, in addition to those directly concerned with formation of polar bodies, in the animal pole topography of eggs. Hibbard and Wintrebert (1928) described a depression and a surrounding concavity in the animal hemisphere of *Discoglossus pictus* (Anura) eggs, which is formed when the egg is in the oviduct and lost soon after fertilization (Denis-Donini and Campanella, 1977). Wilson (1904) found that unfertilized *Dentalium* eggs frequently had a slight depression at the animal pole, just over "a very small disc of clear, dense . . . protoplasm . . ." Schmekel and Fioroni (1975), in their study of the ultrastructure of the zygote and the early segmentation stages of *Nassarius reticulatus,* included a light micrograph depicting the zygote with a slight depression, in which a polar body rests, at the animal pole. Burgess (1977) described a similar slight depression at late metaphase in *Ilyanassa obsoleta.* Judging from the size and shape of the polar lobe in his figure of a thick section and from his statement that most of the work reported was on the second meiotic apparatus, the egg appears to have been in Meiosis II. Of these examples, the *animal dimple* of *Discoglossus pictus* is most similar in form to the Meiosis I pit described here, but is approximately ten times the size of the *Ilyanassa* pit, as is the entire egg.

The surface of the emergent first polar body, with its network of folds, is different from the adjacent surface of the egg. Such a pattern of folds has been found on the surface of the polar lobe, a transient, anucleate protrusion of the vegetal pole, of *Crepidula* and *Buccinum* (Dohmen and van der Mey, 1977). The amorphous, fluffy material on the egg surface may be the remnants of a carbohydrate coat. Dohmen and van der Mey (1977) showed a carbohydrate coat on the surface of the uncleaved egg of *Nassarius reticulatus,* a close relative of *Ilyanassa obsoleta.* Taylor and Anderson (1969) reported a mucopolysaccharide component on the surface of *Ilyanassa* ovarian oocytes on the region exposed to the ovarian lumen, *i.e.,* the vegetal pole. Whether this coat is extended to the animal pole after ovulation has not been shown. In their study of the third polar lobe constriction during first cleavage of *Ilyanassa,* Conrad *et al.* (1973) showed the surface of the fully developed lobe and the two blastomeres are covered with microvilli, with no reticulating folds. To my knowledge, the first polar lobe of *Ilyanassa,* which emerges just after Meiosis I is complete, has not been examined by scanning electron microscopy. However, Dohmen and van der Mey (1977) described a circle of spirally arranged folds at the site of the incipient first polar lobe of *Nassarius reticulatus.* Dohmen and Verdonk (1979) considered two possible relations between these areas of folded surface and morphogenetic localization: they may affect the localization or activation of morphogenetic factors or they may be a reaction of the vegetal cell surface that has no primary significance for development. The observations of this surface pattern reported here for an area of the animal pole that plays no role in development suggests this is a phenomenon that can occur at either pole independently of the localization of cytoplasmic determinants.

One of the most intriguing aspects is the function of the pit. One possibility is that the area functions in fertilization. In *Discoglossus* the bottom of the dimple is the only place at which fertilization can occur (Wintrebert, 1933). Denis-Donini and Campanella (1977) found ultrastructural and biochemical features unique to the surface of the dimple bottom; they speculated that the interaction of the egg and the spermatozoan, which is nearly immotile, is a function of this specialized surface. However, in *Ilyanassa* artificial insemination of ovarian eggs, which lack a pit, is successful and produces normal embryos (author's unpub. obs.; Mirkes, 1972).

Another possibility is that the pit functions as a protective device against mechanical damage to the emerging polar body, damage which could result in cytolysis of the egg. The *Ilyanassa* egg and early developmental stages are exceptionally fragile. They do not survive cutting, injection, nor any breach of the cell membrane.

Another interesting feature of Meiosis I is that the first polar body is produced at an oblique angle to the egg axis. Spiral cleavage is usually defined in terms of the relative positions of the first four blastomeres and their successive quartets of micromeres. The term, therefore, is not applicable to the first cleavage, much less to the meiotic divisions. Conklin (1897) suggested that the first cleavage of *Crepidula,* another spiralian, be described as "*prospectively spiral and dexiotropic.*" Since the spiral pattern is the result of the obliquity of the cleavage planes, the angle of the spindle to the egg axis is a feature more useful for earlier stages, as well as more fundamental and proximate to the cause of spiral cleavage.

Wilson (1904) described the first meiotic spindle as "rotating into a radial position . . ." and presented a figure of this stage that shows the spindle at an angle of about 28° from the egg axis. In *Crepidula* the first meiotic spindle is coincident with the egg axis, but when normal cytoplasmic flows of this egg were suppressed by cold, the spindle assumed an oblique angle (Conklin, 1938). Perhaps, the cytoplasmic flows at that point normally override the basic organization that results in spiral cleavage. To paraphrase Conklin (1897), however, the direction of these spindles may be predetermined, the fact that they are determined is significant.

In my opinion, it seems reasonable that the obliquity of the meiotic spindle and the subsequent angle of extrusion of the first polar body of the *Ilyanassa* egg is an early expression of the organization that later produces spiral cleavage in this embryo.

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